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APPLICATION NUMBER: ***60/455,029***
FILING DATE: ***March 10, 2003***
RELATED PCT APPLICATION NUMBER: *PCT/US04/07232*

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60455029 .031703 PRO

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Sir:

Enclosed please find a provisional application for United States patent as identified below:

Inventor/s (ALL inventors, including NAME, plus city and state of RESIDENCE for each):

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Title: ASSAY DEVICE AND METHOD

including the items indicated:

1. Specification and 79 claims: 3 indep.; 0 dep.; _ multiple dep.
2. [] Drawings, _ sheets (Fig.)
3. [] Assignment for recording to:
4. [] Applicant claims small entity status
5. [X] Check in the amount of $ 160.00

PROVISIONAL PATENT APPLICATION COVER SHEET

If the Commissioner determines that a fee is due, the Commissioner is hereby authorized to charge deposit account No. 04-0100 for any deficiency.

Respectfully submitted,

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Application No. (if known): Not Yet Assigned

Attorney Docket No.: 4359/00000

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Provisional Patent Application Cover Sheet (2 pp)
Specification (24 pp); claims (11 pp)
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ASSAY DEVICE AND METHOD

FIELD OF THE INVENTION

The present invention relates to assays involving specific binding, especially immunoassays. The test devices and methods of the present invention are suitable for quantitative assays as well as for qualitative assays. The invention particularly relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to give an analytical result rapidly and which require the minimum degree of skill and involvement from the user. The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace.

BACKGROUND OF THE INVENTION

Many previous reagent-impregnated test strips used in specific binding assays, such as immunoassays, have required the use of a single labeled specific binding reagent for the analyte and a single unlabeled specific binding reagent for the analyte. The former is located upstream from a detection zone and is mobile, while the latter is located in the detection zone and is permanently immobilized. A sample suspected of containing an analyte is applied to the device. Any analyte in the sample binds with the labeled mobile specific binding reagent and becomes labeled. These labeled complexes travel with the sample downstream to the detection zone where they complex further with the unlabeled, permanently immobilized specific binding reagent. The presence of these complexes in the detection zone indicates the presence of the analyte in the sample. *See, e.g.*, U.S. Patent Nos. 6,228,660 and 6,352,862.

Other earlier sandwich immunoassay devices utilize a colloidal gold labeled

antiligand reagent and antiligand bound solid phase capture particles which are combined with the sample. The reactants are incubated and placed onto a porous film wherein the pore size of the porous film is such that the solid phase particles are retained on the surface of the film but large enough that the unbound colloidal gold reagents can pass through. The particles on the membrane are visually inspected for color to determine the presence or absence of the analyte. *See* U.S. Patent No. 4,853,335.

Another earlier immunoassay device utilizes marking elements and particles which do not affect evaluation. Each of these include bispecific antibodies for the suspected analyte. The device includes a porous catching section that has a pore diameter that is smaller than the diameter of the particles. When the analyte is present, a biochemical reaction takes place between the marking elements and the particles producing a reaction product. The pore size of the catching section is smaller than the size of the articles and smaller than the size of the reaction product. The particles and the reaction product are caught by the catching section, but the marking element can pass through the catching section. Therefore, a visual inspection of the catching section will indicate the presence or the absence of the analyte in the sample. *See* European Patent Application No. EP 0962771.

SUMMARY OF THE INVENTION

The present invention provides a test device for detecting the presence or absence of a selected analyte in a liquid sample. The test device includes a reagent member, a porous carrier, and a detection zone. The reagent member includes a body, a first labeled binding reagent specific for a first binding site of the analyte and a second labeled binding reagent specific for a second binding site of the analye. The first specific binding

site and second binding sites are different. The reagent body is adapted to retain the first and second labeled specific binding reagents when the body and the first and second labeled binding reagents are dry, and to release them when the body and they are moist. The first and second labeled specific binding reagents are capable of forming a first labeled complex with the analyte with the complex including the first labeled specific binding reagent, the analyte, and the second labeled specific binding reagent. The detection zone includes a first porous barrier having an average pore size larger than the diameter of the larger diameter of the first or second labeled specific binding reagent, but smaller than the diameter of the first labeled complex. The detection zone may be a section of the porous carrier or may be separate from and in fluid communication with the porous carrier. The reagent member, porous carrier, and detection zone are arranged so that a fluid applied to the test device would travel sequentially from the reagent member to the porous carrier and to the detection zone. The test device is typically dry before use and moist during use.

The test device may further include a sample receiving member arranged so that a fluid applied to the test device would travel sequentially from the sample receiving member to the reagent member.

The test device may also include a control zone arranged so that a fluid applied to said test device would travel sequentially from the reagent member to the porous carrier, to the detection zone, and to the control zone. The control zone may include a second porous barrier having an average pore size smaller than the diameter of the smaller diameter of the first or second labeled specific binding reagent or an average pore size smaller than the diameter of the larger diameter of the first or second labeled specific

binding reagent. Another type of control zone could include an immobilized binding reagent capable of binding to the first labeled specific binding reagent, the second specific binding reagent, or the first and the second specific binding reagents. The control zone may be a section of said porous carrier or may be separate from and in fluid communication with the porous carrier.

The present invention also provides a test device as described above for detecting the presence or absence of human chorionic gonadotrophin (hCG) in urine. Specific components of one preferred embodiment of a test device for detecting hCG include a first colored latex particle labeled anti-hCG monoclonal anitibody for a first antibody binding site of hCG and a second colored latex particle labeled different anti-hCG antibody specific for a second antibody binding site of hCG, which together with hCG form a first colored latex particle labeled complex. The porous carrier may be a nitrocellulose porous carrier. The detection zone may be a first porous agarose barrier having an average pore size larger than the diameter of the larger diameter of the first or second colored latex particle labeled anti-hCG antibody, but smaller than the diameter of said first labeled complex. Preferably, the colors of the latex particles are visually distinguishable from white.

The present invention also provides a method of detecting the presence or absence of an analyte in a liquid sample by applying the liquid sample to the test devices described herein, whereby at least a portion of any first complexes formed are retained at the detection zone, and at least a portion of the uncomplexed first and second labeled specific binding reagents pass through the detection zone; and (b) detecting the presence

[redacted]

or absence of the first complexes at the detection zone. The presence of the first complexes in the detection zone indicates the presence of the said analyte in the sample.

DETAILED DESCRIPTION OF THE INVENTION

The assay devices and methods of the present invention may be to detect a wide variety of analytes by choosing appropriate specific binding reagents. The analytes can be, for example, proteins, haptens, immunoglobulins, hormones, polynucleotides, steroids, drugs, or infectious disease agents (e.g. of biological, chemical, or biochemical origin). Examples of analytes include, but are not limited to, hCG (human chorionic gonadotrophin) (an hormone whose presence indicates pregnancy), LH (lutinizing hormone) (a hormone whose presence indicates ovulation), infectious disease agents, E-3-G (estrone-3-glucoronide) and P-3-G (pregnanediol-3-glucoronide). The former three are suitably analyzed with the present device in a sandwich assay, while the latter two are suitably analyzed in a competition assay.

The present devices and assays can also be adapted to detect simultaneously or sequentially more than one analyte in a sample, which can also have significant clinical utility. For example, the ratio of the levels of apolipoproteins A_1 and B can indicate susceptibility to coronary heart disease, while the ratio of the levels of glycated haemoglobin (HbA) to unglycated (HbAo) or total (Hb) haemoglobin can aid in the management of diabetes. Test devices may also be configured to measure two steroids simultaneously, such as, for example, E-3-G and P-3-G. A test could also be configured, based upon the detailed disclosure herein, to detect the presence of various different sereotypes of one bacterium, or to detect the presence of soluble serological markers in

humans. For example, a multiple analyte test for the detection of different serotypes of *Streptococcus* can be prepared for groups A, B, C and D. A cocktail of monoclonal antibodies labeled with labels of different diameters, each specific for various pathologically important group serotypes, or a polyclonal antiserum raised against a particular *Streptococcal* group, could be placed onto a reagent member and one or more detection zones properly sized, as described herein, to detect each could be placed on the device.

The reagent member may be one individual reagent sub-member or more than one reagent sub-members each containing one or more specific binding reagents. The reagent member may be composed of, for example, a fibrous material or pad-like structure that may be woven or non-woven, such as, for example, fiberglass pad. Alternatively, the reagent member may be a natural or synthetic porous material such as a macroporous body. The reagent member may be a section of the porous carrier or may be a separate component. The use of a separate component reagent member may facilitate the ease with which the labeled components are taken up by the liquid sample as compared to if the labeled components are incorporated as pre-dosed reagents on the porous carrier. Manufacturing is also facilitated by having the reagent member as a separate component that is placed next to and slightly overlapping the porous carrier during manufacture.

Preferably, the reagent member is in direct moisture-conductive contact with the porous carrier, and the detection zone, whether on the porous carrier or a separate component, is spaced away from the region of contact between the porous carrier and the reagent member. In such an embodiment, the quantity of liquid sample required to saturate the reagent member is preferably not less than the quantity of liquid sample

capable of being absorbed by the mass of porous carrier linking the reagent member and the detection zone. In other words, the liquid capacity of the reagent member is at least equal to the liquid capacity of the working portion of the porous carrier.

Preferably, the reagent member has openings or pores that are at least 10 times greater than the maximum size of the largest label used in the device. Larger pore sizes or openings give better release of the labeled reagent.

The reagent member preferably is not protein-binding or is easily blockable by means of reagents such as BSA or PVA, to minimize non-specific binding and to facilitate free movement of the labeled reagents after the reagent member has become moistened with the liquid sample.

The reagent member can also include surface active agents, such as for example, Tween 20 preferably at about 1%, or solvents, if necessary, to render it more hydrophilic, to promote rapid uptake of the liquid sample, and/or to facilitate the travel of the sample and the reagents though the porous carrier and to minimize or to eliminate any interaction of the reagents or complexes with the porous carrier. Such surface active agents can be incorporated in the solution containing the labeled reagent when this solution is applied to the reagent member during manufacture of the device.

The labeled reagents are preferably incorporated in the reagent member in bulk, e.g., large sheet, form before being subdivided into individual members for use in a test device of the invention. This is the case whether the reagent member is a separate component or a section of the porous carrier. Incorporation of the labeled reagent in a separate reagent member avoids the need to apply labeled reagents in a special zone in the porous carrier, which may need careful pre-treatment, After a solution containing the

labeled reagents has been allowed to saturate the reagent member, the reagent member may be dried, e.g., by vacuum or air-drying, or preferably by freeze-drying. Optionally, the solution can also contain a surface active agent, such as a detergent, and/or a glazing material, such as a sugar, e.g. sucrose. Their presence may enhance release of the labeled reagents and promotes stability of certain specific binding reagents.

Incorporation the labeled reagents in a separate reagent member(s), rather than pre-dosed onto the porous carrier that also incorporates the detection zone, enhances sensitivity of the test, because a substantial quantity of the liquid sample is able to take up the labeled reagents before migrating through the porous carrier to the detection zone. This enhances potential reaction time without significantly increasing overall test time. Also, the liquid which permeates the carrier has a more uniform and consistent composition.

Preferably, the labeled reagents are specific binding partners for the analyte. More preferably, the first and second labeled reagents bind specifically to different sites of the analyte. The labeled reagents and the analyte (if present) cooperate together in a "sandwich" reaction. This results in the labeled reagents being retained in the detection zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte. When the present invetion is used as a test device for pregnancy, the two reagents specific for hCG preferably are monoclonal anti-hCG – clone "MIH 9816" from Seradyn and monoclonal anti-beta hCG – clone "057-10043" from OEM Concepts.

The binding reagents are preferably highly specific antibodies, and more preferably, are monoclonal antibodies, including, but not limited to non-human, chimeric,

and humanized monoclonal antibodies.

The label can be any entity the presence of which can be detected and, preferably, readily detected. Concentration of the label into a small zone or volume should give rise to a readily detectable signal such as, for example, a strongly-colored area. This can be evaluated by eye or by instruments if desired.

Preferably, the label is a direct label, i.e. labels that can be used to produce an instant analytical result without the need to add further reagents in order to develop a detectable signal and/or, in their natural state, are readily visible either to the naked eye when they accumulate, or with the aid of an optical filter and/or applied stimulation, e.g. UV light to promote fluorescence. Preferably, the labels are particulate labels. Such direct particulate labels, e.g. colored latex particles and particularly those colors that are visually distinguishable from white, gold sols, non-metallic colloids, and dye sols, are already known per se. They are robust and stable and can therefore be used readily in a analytical device which is stored in the dry state. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes or surfactants. Preferably, the particulate label is a latex particle, such as a colored latex particle which can be readily visible to the eye if it complexes and is retained in the detection zone or if it is bound in the control zone. If desired, the assay result can be read instrumentally, e.g., by color reflectance or microscopically. Alternatively, the labels can incorporate a fluorescent compound which can respond to applied electromagnetic energy such as ultraviolet light or visible light, to provide an emitted signal that can be measured instrumentally. Preferably, the label is a colored (other than white) latex particle of spherical or near-spherical shape and having a maximum diameter of not greater than

about 2000 micron. More preferably, these particles range in size from about 50 to about 750, and most preferably from about 200 to about 600 microns. Especially preferred are latex particle 250 microns in diameter and 500 microns in diameter. The labels of each specific binding reagent can be of the same or different diameters and of the same or different colors.

Indirect labels, such as enzymes, e.g. alkaline phosphatase and horseradish peroxidase, can be used but these usually require the addition of one or more developing reagents such as substrates before a visible signal can be detected. Such additional reagents can be incorporated in the reagent member, in the porous carrier, or in the sample receiving member if present, such that they dissolve or disperse in the aqueous liquid sample. Alternatively, the developing reagents can be added to the sample before contact with the test device can be exposed to the developing reagents after the complexing reaction has taken place.

Coupling of the label to the specific binding reagent can be by covalent bonding, hydrophobic bonding, or other techniques known in the art. The labeled reagents migrate with the liquid sample as this progresses to the detection zone. Preferably, the flow of sample continues beyond the detection zone and sufficient sample is applied to test device in order that this may occur. Excess labeled reagents which do not participate in forming any first complexes are flushed through the detection zone by this continuing flow.

Various porous materials may be used for the first and/or second porous barriers. For example, different grades of agarose can be used for different pore sizes. Alternatively, pores can be made in suitable materials by laser, drilling, microdrilling, or

photoablation, for example.

If desired, an absorbant "sink" can be provided at the distal end of the test device. The absorbent sink may comprise, for example, Whatman 3MM chromatography paper, and should provide sufficient absorptive capacity. As an alternative to such a sink it can be sufficient to have a length of porous test device which may extend beyond the detection zone.

The presence or intensity of the signal from the first complexes which are retained in the detection zone can provide a qualitative or quantitative measurement of analyte in the sample. A plurality of detection zones arranged in series, through which the aqueous liquid sample can pass progressively, can also be used to provide a quantitative measurement of the analyte, or can be loaded individually with different specific binding reagents to provide a multi-analyte test.

Preferably, the dry porous carrier material comprises a chromatographic strip, such as a strip of nitrocellulose. If desired, the nitrocellulose can be backed with moisture impermeable material, such as polyester sheet. Using nitrocellulose as the porous carrier material has considerable advantage over more conventional strip materials, such as paper, because nitrocellulose has a natural ability to bind proteins without requiring prior sensitisation. No chemical treatment is required which might interfere with the essential specific binding activity of the reagent. Nitrocellulose can be blocked using simple materials, such as polyvinylalcohol. Moreover, nitrocellulose is readily available in a range of pore sizes and this facilitates the selection of a porous carrier to suit particularly requirements such as sample flow rate.

The sample receiving member can be made from any bibulous, porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be unidirectional (ie with pores or fibres running wholly or predominantly parallel to an axis of the member) or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight), polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile and polytetrafluoro-ethylene can be used. It can be advantageous to pre-treat the member with a surface-active agent during manufacture, as this can reduce any inherent hydrophobicity in the member and therefore enhance its ability to take up and deliver a moist sample rapidly and efficiently. Sample receiving members can also be made from paper or other cellulosic materials, such as nitro-cellulose. Materials that are now used in the nibs of so-called fiber-tipped pens are suitable and such materials can be shaped or extruded in a variety of lengths and cross-sections appropriate in the context of the invention. Preferably the material comprising the sample receiving member should be chosen such that it and the reagent member can be saturated with aqueous liquid within a matter of seconds. Preferably, the material remains robust when moist, and for this reason paper and similar materials are less preferred in any embodiment wherein the sample receiving member protrudes from a housing. The liquid must thereafter permeate freely from the sample receiving member into the reagent member.

The control zone can be designed to convey an unrelated signal to the user that

the device has worked. For example, the control zone can be a porous barrier, an antibody that will bind to one or more of the labeled reagents, e.g. an "anti-mouse" antibody if the labeled reagent is an antibody that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip. Alternatively, the control zone can contain an anhydrous reagent that, when moistened, produces a color change or color formation, e.g. anhydrous copper sulphate which will turn blue when moistened by an aqueous sample. As a further alternative, a control zone could contain immobilized analyte which will react with excess labeled reagent(s). As the purpose of The control zone indicates to the user that the sample has proceeded past the detcetion zone and should the control zone should be located downstream from the detection zone in which the desired test result is recorded. A positive control indicator therefore tells the user that the sample has permeated the required distance through the test device.

1. A test device for detecting the presence or absence of a selected analyte in a liquid sample, said test device comprising:

a reagent member comprising a body, a first labeled binding reagent specific for a first binding site of said analyte and a second labeled binding reagent specific for a second binding site of said analyte, wherein said first specific binding site and said second binding site are different; wherein said reagent body is adapted to retain said first and second labeled specific binding reagents when said body and said first and second labeled binding reagents are dry, and to release said first and second labeled specific binding reagents when said body and said first and second labeled specific binding reagents are moist, and wherein in said first and second labeled specific binding reagents are capable of forming a first labeled complex with said analyte, said complex comprising said first labeled specific binding reagent, said analyte, and said second labeled specific binding reagent;

a porous carrier; and

a detection zone comprising a first porous barrier having an average pore size larger than the diameter of the larger diameter of the first or second labeled specific binding reagent, but smaller than the diameter of said first labeled complex;

wherein said reagent member, porous carrier, and detection zone are arranged so that a fluid applied to said test device would travel sequentially from said reagent member to said porous carrier and to said detection zone.

2. A test device as defined in claim 1, wherein said test device is dry.

3. A test device as defined in claim 1 wherein said test device is moist.

4. A test device as defined in claim 1, wherein said detection zone is a section of said porous carrier.

5. A test device as defined in claim 1, wherein said detection zone is separate from and in fluid communication with said porous carrier.

6. A test device as defined in claim 1, further comprising a sample receiving member arranged so that a fluid applied to said test device would travel sequentially from said sample receiving member to said reagent member.

7. A test device as defined in claim 6, wherein said sample receiving member comprises a wick.

8. A test device as defined in claim 1, further comprising a control zone arranged so that a fluid applied to said test device would travel sequentially from said reagent member to said porous carrier, to said detection zone, and to said control zone.

9. A test device as defined in claim 8, wherein said control zone comprises a second porous barrier having an average pore size smaller than the diameter of the smaller diameter of the first or second labeled specific binding reagent.

10. A test device as defined in claim 8, wherein said control zone comprises a second porous barrier having an average pore size smaller than the diameter of the larger diameter of the first or second labeled specific binding reagent.

11. A test device as defined in claim 8, wherein said control zone comprises an immobilized binding reagent capable of binding to said first labeled specific binding reagent, said second specific binding reagent, or said first and said second specific binding reagents.

12. A test device as defined in claim 8, wherein said control zone is a section of said porous carrier.

13. A test device as defined in claim 8, wherein said control zone is separate from and in fluid communication with said porous carrier.

14. A test device as defined in claim 1, wherein said sample comprises a biological sample.

15. A test device as defined in claim 14, wherein said biological sample comprises urine.

16. A test device as defined in claim 14, wherein said biological sample comprises blood.

17. A test device as defined in claim 1, wherein said analyte comprises a protein.

18. A test device as defined in claim 17, wherein said protein comprises a hormone.

19. A test device as defined in claim 18, wherein said analyte comprises human chorionic gonadotrophin

20. A test device as defined in claim 18, wherein said analyte comprises human lutinizing hormone.

21. A test device as defined in claim 1, wherein said reagent body comprises a fibrous material.

22. A test device as defined in claim 22, wherein said regent member comprises a fiber glass pad.

23. A test device as defined in claim 1, wherein said reagent member comprises a porous material.

24. A test device as defied in claim 23, wherein said reagent member comprises a macroporous body.

25. A test device as defined in claim 1, wherein said label of said first labeled specific binding reagent comprises a direct label.

26. A test device as defined in claim 1, wherein said label of said first labeled specific binding reagent comprises an indirect label.

27. A test device as defined in claim 1, wherein said label of sad first labeled specific binding reagent comprises a particle.

28. A test device as defined in claim 27, wherein said label of said first labeled specific binding reagent comprises a gold particle.

29. A test device as defined in claim 27, wherein said label of said first labeled specific binding reagent comprises a latex particle.

30. A test device as defined in claim 29, wherein said latex label comprises a colored latex particle wherein said color is visually distinguishable from white.

31. A test device as defined in claim 1, wherein said label of said second labeled specific binding reagent comprises a direct label.

32. A test device as defined in claim 1, wherein said label of said second labeled specific binding reagent comprises an indirect label.

33. A test device as defined in claim 1, wherein said label of sad second labeled specific binding reagent comprises a particle.

34. A test device as defined in claim 33, wherein said label of said second labeled specific binding reagent comprises a gold particle.

35. A test device as defined in claim 33, wherein said label of said second labeled specific biding reagent comprises a latex particle.

36. A test device as defined in claim 35, wherein said latex label comprises a colored latex particle wherein said color is visually distinguishable from white.

37. A test device as defined in claim 1, wherein said label of said first labeled specific binding reagent and said label of said second specific binding reagent are the same.

38. A test device as defined in claim 1, wherein said labels of said first labeled specific binding reagent and said label of said second specific binding reagent are different.

39. A test device as defined in claim 1, wherein the ratio of diameters of said first labeled specific binding reagent to said second specific binding reagent ranges from about 1:1 to about 1:100.

40. A test device as defined in claim 39, wherein the ratio of diameters of said first labeled specific binding reagent to said second specific binding reagent ranges from about 1:1 to about 1:5.

41. A test device as defined in claim 40, wherein the ratio of diameters of said first labeled specific binding reagent to said second specific binding reagent is about 1:1.

42. A test device as defined in claim 1, wherein said first specific binding reagent comprises a protein.

43. A test device as defined in claim 42, wherein said first specific binding reagent comprises an antibody.

44. A test device as defied in claim 43, wherein said first specific binding reagent comprises a monoclonal antibody.

45. A test device as defied in claim 44, wherein said first specific binding reagent comprises a non-human monoclonal antibody.

46. A test device as defied in claim 44, wherein said first specific binding reagent comprises a chimeric monoclonal antibody.

47. A test device as defined in claim 44, wherein said first specific binding reagent comprises a humanized monoclonal antibody.

48. A test device as defined in claim 44, wherein said antibody comprises an anti-hCG antibody.

49. A test device as defined in claim 1, wherein said second specific binding reagent comprises a protein.

50. A test device as defined in claim 1, wherein said second specific binding reagent comprises an antibody.

51. A test device as defied in claim 50, wherein said first specific binding reagent comprises a monoclonal antibody.

52. A test device as defied in claim 51, wherein said first specific binding reagent comprises a non-human monoclonal antibody.

53. A test device as defied in claim 51, wherein said first specific binding reagent comprises a chimeric monoclonal antibody.

54. A test device as defined in claim 51, wherein said first specific binding reagent comprises a humanized monoclonal antibody.

55. A test device as defined in claim 51, wherein said antibody comprises an anti-hCG antibody.

56. A test device as defined in claim 1, wherein said porous carrier comprises nitrocellulose.

57. A test device as defined in claim 1, wherein said first porous barrier comprises agarose.

58. A test device as defined in claim 9, wherein said second porous barrier comprises agarose.

60. A test device as defined in claim 1, further comprising a casing which contains at least a portion of said test device.

61. A test device as defined in claim 60, wherein said casing has an aperture from which said sample receiving member protrudes.

62. A test device as defined in claim 60, wherein said casing has a transparent or translucent window over at least a portion of said detection zone.

63. A test device as defined in claim 8, further comprising a casing which contains at least a portion of said test device.

64. A test device as defined in claim 63, wherein said casing has an aperture from which a sample receiving member protrudes, wherein said sample receiving member is arranged so that a fluid applied to said test device would travel sequentially from said sample receiving member to said reagent member..

65. A test device as defined in claim 64, wherein said casing has a transparent or translucent window over at least a portion of said detection zone.

66. A test device as defined in claim 65, wherein said casing has a transparent or translucent window over at least a portion of said control zone.

67. A test device as defined in claim 60, wherein said casing further comprises a removable cap adapted to cover said protruding sample receiving member.

68. A test device as defined in claim 63, wherein said casing further comprises a removable cap adapted to cover said protruding sample receiving member.

69. A test device for detecting the presence or absence of human chorionic gonadotrophin (hCG) in urine, said test device comprising:

a reagent member comprising a body, a first colored latex particle labeled anti-hCG monoclonal antibody for a first antibody binding site of said hCG and a second colored latex particle labeled anti-hCG antibody specific for a second antibody binding site of said hCG, wherein said first colored latex particle labeled anti-hCG monoclonal antibody and said second colored latex particle labeled anti-hCG monoclonal antibody binding site are different; wherein said reagent body is adapted to retain said first colored latex particle labeled anti-hCG monoclonal antibody and second colored latex particle labeled anti-hCG monoclonal antibody when said body and said first and second colored latex particle labeled anti-hCG antibodies are dry, and to release said first and second colored latex particle labeled anti-hCG antibodies when said body and said first and second colored latex particle labeled anti-hCG antibodies are moist, and wherein in said first and second colored latex particle labeled anti-hCG antibodies are capable of forming a first labeled complex with said hCG, said first complex comprising said first colored

latex particle labeled anti-hCG antibody, said hCG, and said second colored latex particle labeled anti-hCG antibody;

a nitrocellulose porous carrier; and

a detection zone comprising a first porous agarose barrier having an average pore size larger than the diameter of the larger diameter of the first or second colored latex particle labeled anti-hCG antibody, but smaller than the diameter of said first labeled complex;

wherein said reagent member, porous carrier, and detection zone are arranged so that urine applied to said test device would travel sequentially from said reagent member to said porous carrier and to said detection zone, and wherein aid colors of said latex particles of said first and second colored latex particle labeled anti-hCG antibodies are visually distinguishable from white.

70. A test device as defined in claim 69, wherein said test device is dry.

71. A test device as defined in claim 69, wherein said test device is moist.

72. A test device as defined in claim 69, wherein said detection zone is a section of said porous carrier.

73. A test device as defined in claim 69, further comprising a sample receiving member arranged so that a fluid applied to said test device would travel sequentially from said sample receiving member to said reagent member.

74. A test device as defined in claim 69, further comprising a control zone arranged so that a fluid applied to said test device would travel sequentially from said reagent member to said porous carrier, to said detection zone, and to said control zone.

75. A test device as defined in claim 74, wherein said control zone comprises a second porous barrier having an average pore size smaller than the diameter of the smaller diameter of the first or second colored latex particle labeled specific binding reagent.

76. A test device as defined in claim 74, wherein said control zone is a section of said porous carrier.

77. A test device as defined in claim 69, wherein the ratio of diameters of said first colored latex particle labeled anti-hCG antibody to said second colored latex particle labeled anti-hCG antibody ranges from about 1:1 to about 1:5.

78. A test device as defined in claim 77, wherein said ratio is about 1:1.

79. A method of detecting the presence or absence of an analyte in a liquid sample, said method comprising:

(a) applying said liquid sample to the reagent member of a test device comprising:

a reagent member comprising a body, a first labeled binding reagent specific for a first binding site of said analyte and a second labeled binding reagent specific for a second binding site of said analyte, wherein said first specific binding site and said second binding site are different; wherein said reagent body is adapted to retain said first and second labeled specific binding reagents when said body and said first and second labeled binding reagents are dry, and to release said first and second labeled specific binding reagents when said body and said first and second labeled specific binding reagents are moist, and wherein in said first and second labeled specific binding reagents are capable of forming a first labeled complex with said analyte, said complex

comprising said first labeled specific binding reagent, said analyte, and said second labeled specific binding reagent;

a porous carrier; and

a detection zone comprising a first porous barrier having an average pore size larger than the diameter of the larger diameter of the first or second labeled specific binding reagent, but smaller than the diameter of said first labeled complex;

wherein said reagent member, porous carrier, and detection zone are arranged so that a fluid applied to said test device would travel sequentially from said reagent member to said porous carrier and to said detection zone;

whereby at least a portion of said first complexes formed are retained at said detection zone, and at least a portion of said first and second labeled specific binding reagents pass through said detection zone; and .

(b) detecting the presence or absence of said first complexes at said detection zone, wherein the presence of said first complexes in said detection zone indicates the presence of said analyte in said sample.